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PREFACE

The twenty-sixth meeting of the Laurentian Hormone Conference was held in Mont Tremblant, Quebec, Canada, on August 23 to 29, 1968. The Gregory Pincus Memorial Lecture opened the Conference on Sunday evening with the first of four papers on reproductive endocrinology. The subjects then shifted to proinsulin, to the secretion of hormones by tumors of non-endocrine origin, and to the ultrastructure of the endocrine glands. There were two presentations on the thyroid, two on neuroendocrinology, and three on steroid hormones. The authors deserve especial commendation for the excellence of their papers; the interest of the audience was shown by the vigor of the discussion.

The reader of these proceedings will find not only a considerable amount of new and interesting material but also timely and scholarly reviews on subjects of unusual interest. Year upon year endocrinology seems to expand in one direction or another; this volume bulges with new insights into mechanisms of hormonal secretion and action, and with new concepts and new methods. The membership of the conference is always made up of scientists and clinicians with an ample sprinkling of those who aspire to be both. The program and the discussions reflect this assortment which adds materially to the interest of the conference and to the diversity of the published proceedings. This mixture probably plays a part in the wide readership that these volumes enjoy.

The conference is indebted to Drs. R. Hertz, C. H. Hollenberg, J. C. Melby, W. H. Pearlman, J. A. Pittman, Jr., J. T. Potts, Jr., J. Robbins, M. Saffran, and F. R. Skelton, who served as chairmen of the various sessions, and especially to Miss Joanne Sanford, ably assisted by Mrs. Mina Rano and Miss Jane Woolecombe, for the expert secretarial conduct of the meeting.

Valuable financial assistance was provided by members of the pharmaceutical industry, and the committee would like to express their thanks to the following companies for their contributions: Abbott Laboratories; Armour Pharmaceutical Company; Ayerst Laboratories; Ciba Pharmaceutical Company; Hoffman-La Roche Inc.; Lederle Laboratories; The Lilly Research Laboratories; Mead Johnson Research Center; Merck Sharp & Dohme Research Laboratories; The Wm. S. Merrell Company; Organon; Ortho Research Foundation; Parke, Davis & Company; Chas. Pfizer & Co., Inc.; Schering, A. G.; Schering Corporation; G. D. Searle & Co.; Smith Kline & French Laboratories; Smith, Miller and Patch, Inc.; The Squibb Institute for Medical Research; Sterling-Winthrop Research Institute; Syntex

Corporation; The Upjohn Company, Warner-Lambert Research Institute; and Wyeth Laboratories Inc. With their help we were able to have with us Dr. W. D. Alexander of the University of Glasgow as a guest lecturer.

E. B. ASTWOOD

Boston, Massachusetts
July, 1969

CONTENTS

A Model for the Regulation of Ovulation in the Rat[1]

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I. Introduction

A. The Problem

Our specific area of interest is the mechanism(s) by which reproductive cyclicity is produced and maintained in the female mammal. The evidence that reproduction manifests a cyclic or periodic nature is abundant (Asdell, 1964). This cyclicity appears to have a high degree of adaptive significance: the life span of ovarian follicles, once gonadotropin stimulation of a given set begins, is severely limited, being terminated either by ovulation or atresia. The ovulated ova themselves have a life span of less than 24 hours in the oviduct. If fertilization does not take place, or if atresia of the stimulated follicles occurs, a new set of follicles must be stimulated. This lends an intrinsically cyclic nature to ovarian function and makes it imperative that a set of linkages take place between the female organism and the external world, primarily to synchronize the behaviors of male and female animals so that mating may take place at a time when fertilization is possible. Other necessities (like predator-prey relationships, food-gathering activities, or display for population control) also dictate that the processes of female reproduction be linked to geophysical periodicities, such as the 24-hour light-dark cycle or seasonal shifts (Amoroso and Marshall, 1960; Wynne-Edwards, 1962). The problem of the present paper is to review what we know of how the cyclicity is maintained in the rat.

B. The Approach

Kuhn (1964) has postulated that a scientific revolution occurs when scientists put forward a new "paradigm" which provides a different and more meaningful way of looking at data in a particular field. The notion of "endocrine organ and hormonal secretion" is such a paradigm, much as the concept of "neuroendocrine processes" appears to be a newer paradigm. Another paradigm, which has influenced many fields of engineering and biology, is that of the "control systems approach," which has provided a new way of viewing problems in many areas. We have chosen to use this kind of approach in examining the problem of cyclicity in the rat.

Recently a number of biologists and systems analysts have addressed them-

[1] The Gregory Pincus Memorial Lecture.

selves to the application of systems analysis to biological problems. Particularly useful to this writer have been the following references: Mesarovic (1968), Yates *et al.* (1968), Yamamoto and Raub (1967), Iberall and Cardon (1964), Hare (1967), Gann *et al.* (1968), Schoeffler *et al.* (1968), Grodins (1963), and Milhorn (1966). The approach encompasses a number of stages, which are carried out simultaneously: (1) identify from extant experimental data the essential variables and connecting linkages among the system components; (2) make a model of the system; (3) simulate the system by computer and/or mathematical equations; (4) perform "experiments" on the model to verify its resemblance to the real system and to predict the results of new experiments on the real system; (5) perform these experiments and modify the model to conform to the behavior of the real system, etc. The present paper describes our attempts to apply this approach to that neural-pituitary-ovary-target organ system in the female rat which produces the remarkable sequence of events called the "estrous cycle." The still primitive quality of the attempt described in this paper can be seen by contrasting the present analysis with the elegant analysis of the adrenocortical control system by Yates *et al.* (1968).

II. Selected Experimental Observations

Selection of the experimental observations to be reported was made on the basis of presumed relevancy to the problem of explaining the rat estrous cycle. (1) The time domain of interest was limited to the 4-day or 5-day cycle in an adult female rat. Thus data concerning ontogenetic features such as the early effect of androgen (Barraclough, 1966), puberty (McCann and Ramirez, 1964), or aging will not be reviewed or incorporated in the model. Pregnancy and pseudopregnancy will not be considered except insofar as they might shed light on the control of the estrous cycle. (2) The phenomenological domain of interest will be restricted to the whole cell, organ, or organism level. Questions of the mechanism of action of gonadotropic hormones on ovarian cells to produce steroids or the mechanism(s) of action of steroids on uterine cells to produce growth or on neural cells to alter behavior, will not be considered; these processes will be treated as "black boxes," and only the inputs and outputs of the boxes will be considered (Mesarovic, 1968). (3) Certain other questions which have been of great interest to endocrinologists will not be considered in detail. These include the issues of the site of the receptors for steroids for controlling behavior and gonadotropin release (Bogdanove, 1964) and the specific nature of the communicating network between the pituitary and the hypothalamus (Greep, 1963).

A. Delineation of the Manifest Events of the Estrous Cycle

The major ovarian, uterine, and vaginal events of the rat estrous cycle were delineated by Long and Evans in their classical monograph (Long and Evans, 1922). Their observations are summarized in Table I. The cycle

TABLE I

Definition of Estrous Cycle Stages According to Long and Evans (1922)

Stage	Vaginal smear	Uterus	Ovary and oviduct	Duration of stage (hours) Mean ± SD	CV[a]
One (pro-estrus)	Epithelial cells	Distention starts	Large follicles	14.3 ± 5.2	36%
Two[b] (early estrus)	Cornified cells	Greatest distention, and then regression	Largest follicles; eggs may start maturation	38.2 ± 13.6	36%
Three (late estrus)	Cornified cells	Uterus regressed	Ovulation		
Four (met-estrus)	Cornified cells plus leukocytes	Uterus regenerating	Young CL[c]; eggs in oviduct	7.4 ± 4.4	56%
Five (di-estrus)	Leukocytes plus epithelial cells	Uterus regenerating	Eggs in oviduct; CL growing	56.1 ± 11.4	20%

[a] CV = coefficient of variation (SD/mean × 100).
[b] Animal in heat.
[c] CL = corpora lutea.

was perceived by Long and Evans, and by many later investigators, as essentially a continuous sequence of events, with a wide variation among animals and cycles in the length of the various stages, as defined by vaginal events (Table I). Even in the early data of Long and Evans, however, several indications were available suggesting that the rat estrous cycle was not just a continuously unfolding process stretched out over a variable number of hours. First, there was the observation that 71% of the cycles observed were *either* 4 days or 5 days in length, not intermediate; second, there was the statement that "ovulation may occur at any time in the twelve-hour interval embraced from the eighteenth to the thirtieth hour after Stage One"; third, was the statement that "Oestrus (behavioral) may be exhibited as early as three hours before the appearance of cornified cells or as late as twenty hours

after" (Long and Evans, 1922). As well as can be deduced from the monograph, the authors did not associate the variability in times of ovulation and mating behavior with respect to cornification to the difference between 4-day and 5-day cycles.

Astwood (1939) measured uterine weight and intraluminal water throughout the estrous cycle in a group of rats showing either 4- or 5-day cycles and demonstrated that maximal luminal fluid was seen during the proestrous-estrous vaginal smear conversion. He mentioned the variability of uterine weight associated with the variation in length of the diestrous interval from animal to animal. His data clearly indicated the profound influence of cycle stage on the uterus. These observations have been confirmed by Mandl (1952), Schwartz (1964), and others.

Changes in ovarian follicle size during the estrous cycle were demonstrated by Boling *et al.* (1941) and Mandl and Zuckerman (1952). A linear increase of the volume of a given set of follicles occurs during the cycle, starting with the beginning of heat in the previous cycle; the final follicular volume (before preovulatory swelling) is greater in 5-day cycles than in 4-day cycles. With the onset of the next heat, preovulatory swelling starts and the follicles grow rapidly until ovulation supervenes about 6–12 hours later. Blandau *et al.* (1941) showed that in 75% of rats the onset of heat occurred between 4 PM and 10 PM, and lasted about 14 hours. In these studies (Boling *et al.*, 1941; Blandau *et al.*, 1941; Young *et al.*, 1941) the vaginal smear was not used as an important criterion of cycle stage, and a better correlation was seen between the time of heat and ovulation than was apparent in the studies of Long and Evans (1922). This appears to be the case because of the separation of animals of different cycle lengths, emphasis being placed on the day when both heat and ovulation occur.

The more recent studies of Everett and Sawyer on the rat estrous cycle revealed the missing evidence which provided the means of bringing together the various apparently contradictory data on correlations among the vaginal smear, heat, and ovulation. Everett (1948) pointed out that ovulation occurs during the third night in rats running 4-day cycles, and on the following night in the 5-day cyclers; in the latter animals vaginal cornification is prolonged. Referring to the time of ovulation and vaginal estrus Everett (1961) has said, "In the rat some authors have placed it (ovulation) early (Young *et al.*, 1941) and others late (Long and Evans, 1922) with respect to vaginal estrus. In the writer's colony, both relations hold in 4-day and 5-day cycles, respectively." Everett and Sawyer demonstrated, by the use of blocking drugs (Everett *et al.*, 1949; Everett and Sawyer, 1950, 1953) or hypophysectomy (Everett, 1956), that the LH[1] release responsible for cyclic ovulation occurred after 2 PM and before 4 PM on the afternoon of proestrus

in the 4-day rat, and over essentially the same "critical period" 1 day later in the 5-day rat. Figure 1 places on the same time scale all the events we have been discussing. (The light-dark schedule shown is that used by Everett and Sawyer).

Further studies revealed that in the 4-day rat, blocked on the day of proestrus by pentobarbital administered at 2 PM, ovulation was delayed a full

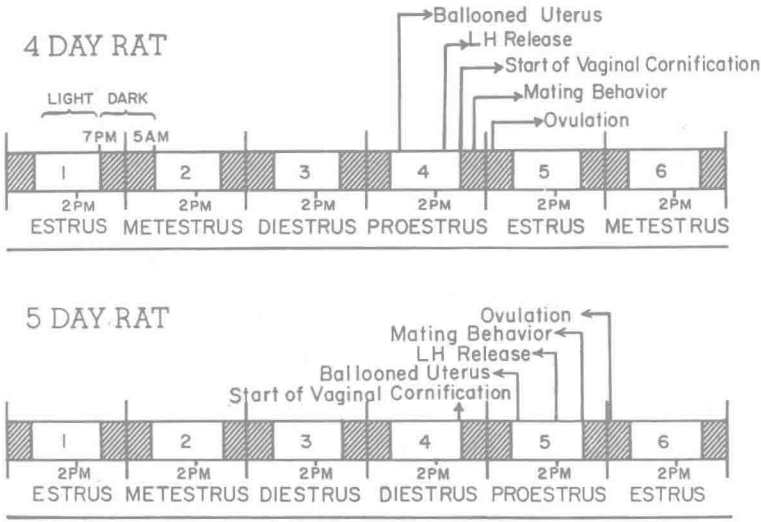

FIG. 1. The timing of the manifest events of the rat estrous cycle. Adapted from Schwartz (1969).

24 hours and could again be blocked on day 5 (expected estrus, Fig. 1) (Everett and Sawyer, 1950). Progesterone could induce ovulation 24 hours early, when given on day 4 in the 5-day rat (Everett and Sawyer, 1949), and this advancement could be blocked by atropine given at 2 PM. These data suggested that a 24-hour periodicity in the facilitating mechanism for LH release existed; the unrecognized existence of this discontinuity, which meant that ovulation could occur only at a given time during the 24-hour day, was the source of the conflict in the literature regarding the timing of various events. If the timing of estrogen secretion is fairly variable, with the vaginal smear responding to estrogen, but the timing of LH release and ovulation is responding to a time of day signal (as well as steroid, see below), then comparisons between 4- and 5-day rats must take into account the time of release of the LH for ovulation, not the condition of the vaginal smear (Fig. 1). On the day of "proestrus," selected for the occurrence of mating behavior and LH release, the morning vaginal smear may be nucleated,

fully cornified, or even leukocytic (Table I, Fig. 1). Using the vaginal smear as the *sole* criterion for estrous events is thus misleading and should be discontinued in favor of following the smear pattern for several cycles and determining for a given colony when the day of proestrus (LH release for ovulation) occurs. Many investigators still adhere to the Long and Evans classification (Table I), which makes interpretation of their results difficult (Wurtman *et al.*, 1963); Kopin and Wurtman, 1963; Boccabella and Alger, 1967; Hamilton *et al.*, 1967).

B. Time Mapping of Some Essential Variables during the Estrous Cycle

Having delineated the manifest events (Fig. 1), to be used as a basis for properly identifying the days of the estrous cycle, we can proceed to examine more recent evidence on the sequence of changes in essential variables during the cycle. A summary in graphical form of some of the data in a 4-day cycle appears in Fig. 2.

The follicular changes seen in Fig. 2 were described in the preceding section; the drop in volume represents ovulation (Everett, 1961). Estrogen secretion rate in Fig. 2 is a theoretical curve based on the timing of blockade of estrogen effects (Schwartz, 1964; Schwartz and Talley, 1965; Shirley *et al.*, 1968; Schwartz and Ely, 1969). These experiments will be described in the next section. Indirect support of the contention that estrogen secretion increases on the day before proestrus is also seen in the increase of follicular Δ^5-3β-hydroxysteroid dehydrogenase content at that time (Pupkin *et al.*, 1966). The progesterone secretion curve shown is from Hashimoto *et al.* (1968), but similar data have been reported by Eto *et al.* (1962), Telegdy and Endroczi (1963), and Feder *et al.* (1967). Unfortunately none of these investigators reported *when* this rise occurs relative to the onset of the critical period for LH release. Data relative to this point will be presented later in this section and in the next.

LRF content in the hypothalamus (Ramirez and Sawyer, 1965) drops abruptly on the afternoon of proestrus, as do LH and FSH content in the pituitary (Mills and Schwartz, 1961; Schwartz and Bartosik, 1962; Schwartz and Calderelli, 1965; McClintock and Schwartz, 1968; Caligaris *et al.*, 1967; Goldman and Mahesh, 1968). Increased levels of LH and FSH appear in the plasma during the critical period (McCann and Ramirez, 1964; Schwartz and Calderelli, 1965; McClintock and Schwartz, 1968), indicating an increase in rate of release of these gonadotropins; this release can be prevented by barbiturate injection before 2 PM of proestrus (Schwartz and Calderelli, 1965; McClintock and Schwartz, 1968).

Quite recently Miyake (1968) has published data relating pituitary and

plasma LH contents to ovarian vein progesterone and estrogen (by bioassay) levels (Fig. 3). Two points are of particular interest: (1) the directly measured estrogen curves look remarkably as predicted from indirect evidence (Fig. 2); (2) estrogen secretion falls abruptly and progesterone secretion rises abruptly *after* the surge of LH release, not before.

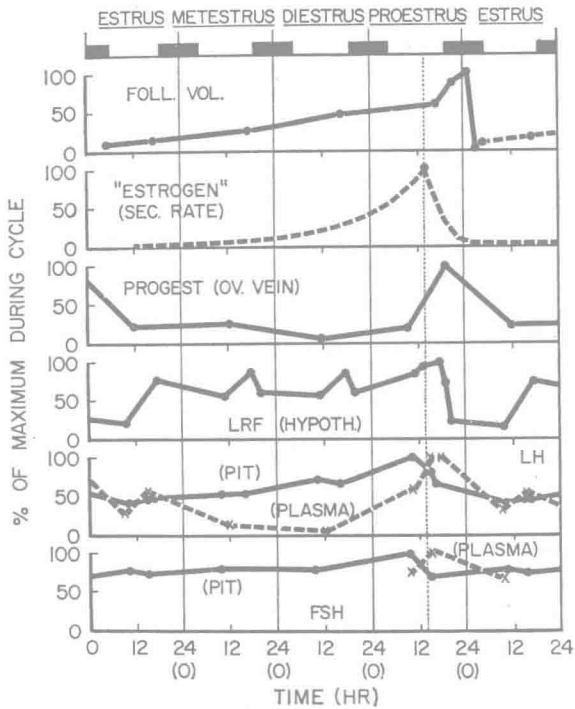

FIG. 2. A number of variables seen on the time scale of the 4-day cycle. The vertical dotted line on the day of proestrus denotes 2 PM. The data have been recalculated, on the basis of the maximum (100%) seen during the cycle, from the following references: follicle volume (Boling *et al.*, 1941); "estrogen secretion rate" (Schwartz, 1964; Schwartz and Talley, 1965; Shirley *et al.*, 1968); progesterone in ovarian vein blood (Hashimoto *et al.*, 1968); LRF in the hypothalamus (Ramirez and Sawyer, 1965); LH in the pituitary and plasma (McCann and Ramirez, 1964; Schwartz, 1964; Schwartz and Bartosik, 1962; Schwartz and Calderelli, 1965); FSH in pituitary and plasma (McClintock and Schwartz, 1968). Reproduced from Schwartz (1969) by permission.

In addition to the reasonably direct evidence of changes seen in Figs. 2 and 3, there is some ancillary evidence obtained in untreated animals which further contributes to our knowledge of the proestrous progesterone secretion. Both Armstrong (1968) and Astwood (1939) have adduced strong evidence suggesting that progesterone secretion is at least partly responsible for the

loss of intraluminal water and the drop in uterine weight which occurs between the days of proestrus and estrus (Long and Evans, 1922; Mandl, 1952; Schwartz, 1964). The termination of estrogen secretion (Figs. 2 and 3) may also contribute to this uterine change (Schwartz, 1964; Schwartz and Gold, 1967; Shirley *et al.*, 1968).

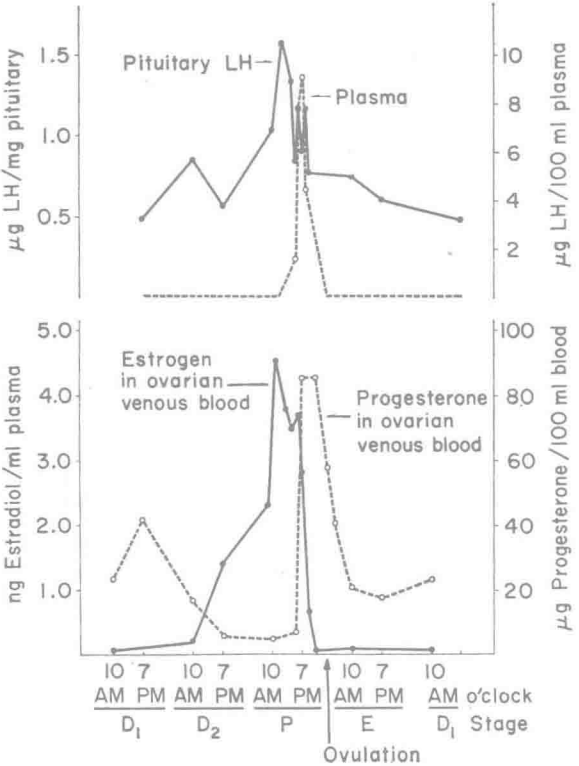

FIG. 3. Cyclic fluctuations of pituitary and plasma LH, and estrogen and progesterone in ovarian venous blood in 4-day cyclic rats. Lights on from 8 AM to 8 PM. Reproduced from Miyake (1968) by permission.

Because of the conflicts regarding when behavioral heat occurs with respect to other events of the cycle (Long and Evans, 1922; Blandau *et al.*, 1941; Young *et al.*, 1941), we have reinvestigated this point, placing it in the context of the new viewpoint on the estrous cycle provided by the work of Everett and Sawyer (Everett, 1961). The data (Lorenzen-Nequin and Schwartz, 1967) are summarized in Fig. 4. No evidence of mating behavior was seen in 4-day cyclers before 2 PM, but half of the 5-day cyclers had already responded by the 2 PM test period. The average time of response (and

standard deviation) for the 4- and 5-day rats, respectively, was 4:41 PM ± 1 hour, 19 minutes ($N = 26$) and 2 PM ± 2 hours, 52 minutes ($N = 14$). These average times and the variances are significantly different from each other. Thus, the data indicate a highly predictable time of heat on the day of proestrus, when this is properly defined by other events (Fig. 1). The possible relationship of this finding to progesterone secretion will be discussed in the next section.

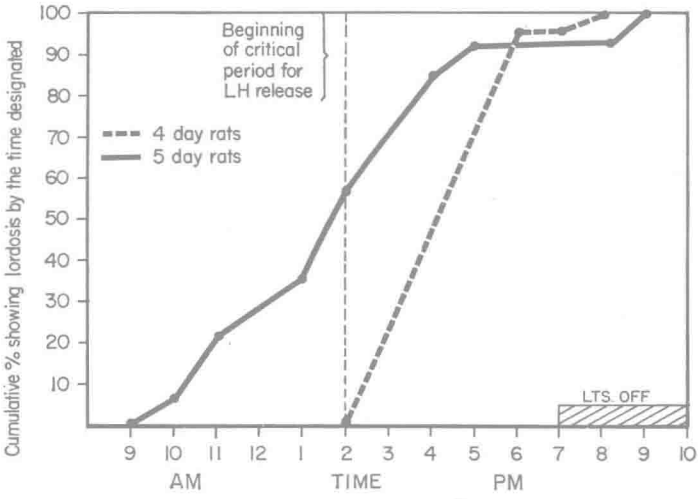

FIG. 4. Cumulative percentage of rats demonstrating lordosis, seen as a function of time of day on proestrus. Lights on from 5 AM to 7 PM. The females were randomly paired for 10 minutes with a male at hourly intervals, starting at 8 AM and continuing until a positive response occurred (three lordosis responses to three mountings). These data have been presented in preliminary form (Lorenzen-Nequin and Schwartz, 1967).

C. The Introduction of Perturbations into the Estrous Cycle

The emphasis in this section will be on acute experiments, performed at different stages of the estrous cycle, which have yielded information about the causative sequence of events.

1. Removal of Endogenous Hormones

An exceedingly useful technique has been that of removing endogenously secreted hormones abrutly by a variety of techniques and then following the short-term effects. A broad summary of results of procedures applied on the day of proestrus is seen in Table II. Since ovariectomy at proestrus did not itself block the estrous vaginal cornification (Table II), it is not surprising that the other procedures also did not do so. Hypophysectomy or

barbiturate injection before 2 PM blocks ovulation and LH discharge from the pituitary (Schwartz and Calderelli, 1965). By contrast, injection of an anti-ovine-LH serum blocks ovulation, without preventing LH release from the pituitary. Barbiturates block only for 24 hours, but the anti-LH serum blocks for a full cycle. An anti-ovine-FSH serum, which was capable of inhibiting the effects of rat pituitary FSH in the Steelman-Pohley assay, did not block ovulation. However, in some rats it exerted a delay in the appearance of cornification and ovulation in the next cycle. The results in Table II

TABLE II

Experimental Procedures Tested on Day of Proestrus[a]

	Events at estrus[b]					
Procedure	Time	Vagina	Ova	Pituitary LH	Next cycle	Reference
None	—	C	Yes	Low	Normal	Figs. 1–3
Ovariectomy	10 AM	C	—	Low	None	Schwartz (1964)
Hypophysectomy	2 PM	C	No	—	None	Everett (1956)
Hypophysectomy	3 PM	C	Yes	—	None	Everett (1956)
Barbiturates	2 PM	C	No	High	Ova, 24 hr	Everett and Sawyer (1950); Schwartz (1964)
Anti-LH serum	1 PM	C	No	Low	Delay	Schwartz and Gold (1967); Schwartz and Ely (1969)
Anti-FSH serum	1 PM	C	Yes	Low	Delay (variable)	Schwartz and Ely (1968)

[a] Day 4 in 4-day rats; day 5 in 5-day rats.

[b] Next morning. C = cornification.

indicate that by 10 AM on the day of proestrus the ovary has contributed the steroid secretion necessary for LH release, ovulation, and estrous vaginal cornification.

The same perturbations were applied on the day before proestrus (Table III). Either ovariectomy or an estrogen antagonist, MER-25, block vaginal cornification, LH release, or ovulation if given early enough on this day. (A more detailed summary of the MER-25 data is given in Table IV.) If ovariectomy, or MER-25, is delayed until late enough on the day before proestrus, signs of estrogen secretion occur (Tables III and IV). The work of Lawton and Sawyer (1968) indicated in 4-day rats that hypophysectomy has to be performed earlier than ovariectomy to accomplish blockade of estrogen secretion on the day before proestrus (Table III). Barbiturate injection on this day did not block estrogen secretion, and most of the rats